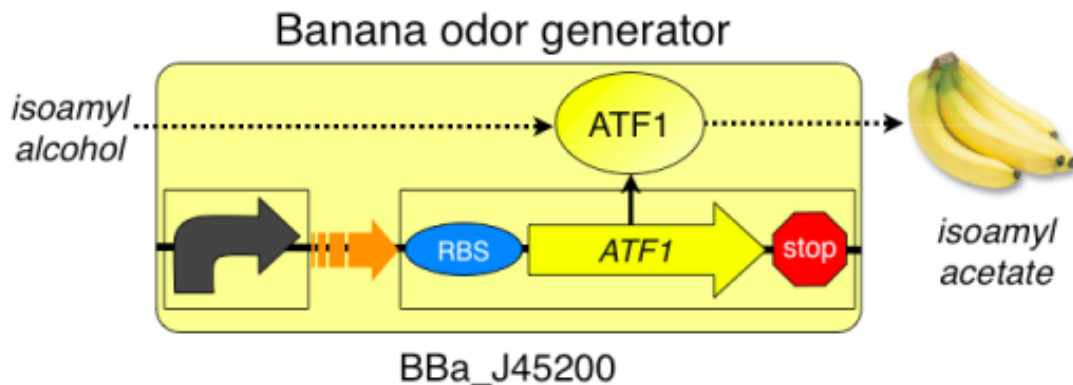


BioBuilding: Synthetic Biology for Students: Eau that Smell

LAB 1: Eau that smell --Protocol B

- Compare 2 competing designs to optimize system performance



Protocol B

You teacher will inform you if you need to conduct the Day 1 or Day 2 procedures.

Day 1

We will be receiving our bacterial strains with the plasmids already inserted. The strains may come in the form of a "stab" or "slant," a test tube with a small amount of bacteria on a slanted media, in which case you will have to streak out the bacteria onto a petri dish to continue the experiment. If the bacteria have arrived on petri dishes, you can proceed to **Day 2**.

1. Using a sterile toothpick or inoculating loop, gather a small amount of bacteria from the stab and transfer it to a petri dish containing Luria Broth (LB) agar plus ampicillin medium.
2. Repeat with the remaining stab samples, streaking out each onto a different petri dish.
3. Place these petri dishes media side up in a 37°C incubator overnight.

A video of this procedure is [here](#).

Day 2:

1. Using a sterile inoculating loop or toothpick or pipet tip, transfer a bacterial colony from one of the petri dishes to a large sterile culture tube containing 3 ml of Luria Broth (LB) + ampicillin. This volume is more than enough for each strain that each student or team of students must grow.
2. Repeat for each strain you will inoculate.
3. Place the culture tubes in the roller wheel in the incubator at 37°C overnight. Be sure to balance the tubes across from each other to minimize stress on the roller wheel. Alternatively, tubes can be placed on a platform shaker to grow the cells with aeration overnight.

A video of this procedure is [here](#).

BioBuilding: Synthetic Biology for Students: Eau that Smell

Day 3: Procedure if using a spectrophotometer

A video of this procedure is [here](#).

1. Prepare a stock growth solution with
 - 300 ml Luria broth
 - 300 μ l Ampicillin (final concentration 100 mg/liter)
 - 250 μ l isoamyl alcohol
2. Mix this stock growth solution, by swirling the bottle or vortexing gently.
3. Set aside 2 ml of this mixture for each student group into a small sterile culture tube. This aliquot will serve as the blank for the spectrophotometer.
4. Move 75 ml of the broth solution to 125ml sterile erlenmeyer flask and add 2ml of bacteria from one of the overnight cultures, e.g. strain 1-1.
5. Repeat the addition of 2ml of bacteria to 75 ml of broth in the erlenmeyer flasks for each of the overnight cultures.
6. Add a stir bar to each culture flask and place onto stir plates. Stir slowly. Cover the flasks with foil.
7. Remove 2 ml from each sample to read the starting density of each. If you are testing all 4 samples you should now have 5 small test tubes (4 with bacterial dilutions and one blank).
8. Prepare the spectrophotometer by setting it to OD600.
9. Note the time and take an "initial" density reading for the bacterial samples. Please note that your teacher may have carried out the preceding steps in advance of the lab. If that is the case, the teacher will tell you how much time has elapsed. That time will be your T_0 .
10. After 20 minutes, move 1 or 2 ml from each sample to cuvettes. Note: the volume you use here will depend on the size of the cuvette appropriate for your spectrophotometer. Please follow the teacher's instructions.
11. Read the blank and adjust the % Absorbance to zero.
12. Read the sample tubes and record the % Absorbance.
13. Sniff the flask for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and the cultures before sniffing. Record your data.
14. At 20 minute intervals repeat steps 11-14.
15. Between time points, you can calculate the bacterial population: 1 OD600 unit = 1×10^9 bacteria.

BioBuilding: Synthetic Biology for Students: Eau that Smell

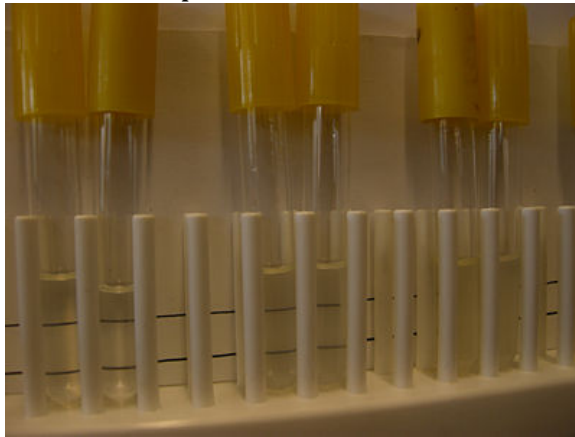
Day 3: Procedure, if no spectrophotometer is available

The turbidity of the bacterial populations can be estimated using the [McFarland Turbidity Scale](#). This method uses suspensions of a 1% BaCl₂ in 1% H₂SO₄ that are visually similar to suspensions of various populations of *E. coli*.

Preparations of McFarland Standards:

Note: Your teacher may tell instruct you to do this in advance of the labs.

1. Following your teacher's instructions, obtain small clear test tubes containing the turbidity standards. The tubes should contain enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom. Make sure each tube is properly labeled with its turbidity standard number. If you are filling the tubes from stock bottles of the standards, use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
2. Place the samples in a test tube rack that allows you to view them from the side. Use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
3. On a blank index card or paper use a marker to draw two thick black lines. These lines should be within the height of the standards.
4. Place the card with the lines behind the standards.



Measuring population growth and banana smell:

1. Prepare a stock growth solution with
 - 300 ml Luria broth
 - 300 µl Ampicillin (final concentration 100 mg/liter)
 - 250 µl isoamyl alcohol
2. Mix this stock growth solution, by swirling the bottle or vortexing gently.
3. Move 75 ml of the broth solution to 125ml sterile erlenmeyer flask and add 2ml of bacteria from one of the overnight cultures, e.g. strain 1-1.
4. Repeat the addition of 2ml of bacteria to 75 ml of broth in the erlenmeyer flasks for each of the overnight cultures.
5. Add a stir bar to each culture flask and place onto stir plates. Stir slowly. Cover the flasks with foil.
6. Remove 2 ml from each sample to read the starting density of each. If you are

BioBuilding: Synthetic Biology for Students: Eau that Smell

- testing all 4 samples you should now have 4 small test tubes (One for each strain). Replace the foil and keep each flask spinning slowly.
7. Note the time and take an "initial" density reading for the bacterial samples. (Please note that your teacher may have carried out the preceding steps in advance of the lab. If that is the case, the teacher will tell you how much time has elapsed.) That time will be your T_0 . To take this initial reading, you will compare your cultures to the standards. To compare your bacterial cultures to the standards, you will need to place the bacterial sample in a test tube of the same size and equal volume as the standards. Be sure to label these sample tubes.
 8. Place the sample tube next to the standard tubes. You should move the sample to compare it to the standard tubes with the most similar turbidity. You can make this assessment more precise by looking for a standard that most similarly obscures the black lines on the background card.
 9. Use the table below to determine the comparable OD 600. 1 OD 600 unit equals approximately 1×10^9 cells.
 10. After 20 minutes repeat steps 6-9, but record the time as $T_0 + 20$.
 11. Sniff the flask for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and the cultures before sniffing. Record your data.
 12. At 20 minute intervals repeat steps 6-11. Record time as $T_0 +$ the number of minutes since T_0 .
 13. Your teacher will inform you of the length of this test and may provide you with data from other classes.

Turbidity Scale	OD 600	1% BaCl₂/1% H₂SO₄ (mL)
0	0	0.0/10
1	0.1	0.05/9.95
2	0.2	0.1/9.9
3	0.4	0.2/9.8
4	0.5	0.3/9.7
5	0.65	0.4/9.6
6	0.85	0.5/9.5
7	1.0	0.6/9.4

BioBuilding: Synthetic Biology for Students: Eau that Smell

Data Table

In your lab notebook, you will need to construct a data table as shown below for each of the samples.

SAMPLE _____

Time (minutes from T ₀)	OD 600	cells/ml*	Banana smell (0-6)
T ₀ (initial)			
T ₀ +			
T ₀ +			
T ₀ +			
T ₀ +			
T ₀ +			
T ₀ +			
T ₀ +			

■ 1 OD600 unit ~ 1×10^9 bacterial cells/ml